

PATENT  
Attorney Docket No. 24747-1104

Amendments to the Specification:

Please replace the paragraph on page 23, lines 5-17, with the following paragraph:

Previously, Grkovic et al. (1995) have shown that the pADK-13 mutation can be complemented with the pADAP 11 kb *HindIII* fragment (pGLA-20). However, the pADK-10 mutation was unable to be complemented with pGLA-20. In an attempt to isolate the region that may complement the pADK-10 mutation the previously described pGLA-20 derived, pADK-35 null mutation (Grkovic et al. 1995) was used as a selective marker (Fig 1), to select the *BgIII* fragment encompassing both the pADK-10 and pADK-35 mutations. pADK-35 DNA was isolated and digested with the restriction enzyme *BgIII*. The resultant digest was ligated into the *BamHI* site of bBR322 to form the construct pBG35 (containing 12.8kb *BgIII* - mini-*Tn10* fragment). pBG35 was placed separately in *trans* with pADK-10 and pGLA-20, and the resultant strains bioassayed against grass grub larvae. Results showed that pBG35 was able to complement the pADK-10 mutant, but was unable to induce any symptoms of amber disease when placed in *trans* with pGLA-20, indicating that there must be another region on pADAP needed to induce amber disease.

Please replace the paragraph on page 36, lines 10-24, with the following paragraph:

Using the polymerase chain reaction (PCR) the initiation codons ATG of the three *sep* genes (*sepA*, *sepB* and *sepC*) were individually placed into the unique *NdeI* site (restriction enzyme site (CATGG) of the HIS-tag arabinose expression vector pAV2-10 (obtained from Chuck Shoemaker - AgResearch). Because large proteins i.e. greater than 50 kda are limited in their ability to bind to HIS tag affinity columns the carboxyl terminus of each of the Sep proteins did not need to be in frame with the HIS-tag site. Instead wild type DNA (non PCRd) containing a downstream chloramphenicol resistance gene was ligated into the appropriate restriction enzyme site (*sepA*, *SunI*; *sepB* *HindIII*; *sepC* *BstXI*) of the pAV2-10-*sep* derived vectors:-

-the use of the chloramphenicol resistant marker provided by the vector pACYC184 enhances the stability to each of the expression constructs i.e. -the antibiotic ampicillin to which the pAV2-10 is resistant too is cleaved in the media to an inactive form leading to possible plasmid free segregants